

Applicants: Gloria C. Li and Paul W.J.J. Burgman
Serial No.: Not Yet Known 10/12,642 BB
Filed : Herewith
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Amendments to the Specification

amend following paragraph
On page 1, ~~before line 1, please insert the sentence:~~

BB 6-11-09

This application is a continuation of U.S. Serial No. 09/750,410, filed December 28, 2000, a continuation of PCT International Application No. PCT/US99/14702, filed June 30, 1999, on behalf of Sloan Kettering Institute for Cancer Research, claiming priority of U.S. Provisional Application No. 60/091,181, filed June 30, 1998, the contents of which are hereby incorporated into this application by reference.

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the following text:

The genotype of the mice was first determined by tail PCR analysis which distinguishes endogenous from the targeted *Ku70* allele, and subsequently confirmed by Southern blot analysis. The PCR reaction contained 1 µg genomic DNA; 0.6 µM (each) of primers HO-2: GGGCCAGCTCATTCCTCCACTCATG (SEQ ID NO: 24), HO-3: CCTACAGTGTACCCGGACCTATGCC (SEQ ID NO: 25) and HO-4: CGGAACAGGACTG-GTGGTTGAGCC (SEQ ID NO: 26); 0.2 mM (each) dNTP; 1.5 mM MgCl₂ and 2.5 U of Taq polymerase. Cycling conditions were 94°C for 1 min,

On page 25, lines ⁷8-20, please delete the paragraph which begins "To confirm that the disruption of *Ku70* . . ." and insert the following paragraph:

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To confirm that the disruption of *Ku70* produces a null mutation, *Ku70* protein expression was measured by Western blotting using polyclonal antisera against intact mouse *Ku70*. The lack of *Ku70* was also verified by a *Ku*-DNA-end binding assay (gel mobility shift analysis). Cell extracts were prepared and gel mobility shift assays were performed as described (22). Equal amounts of cellular protein (50 µg) from *Ku70*^{+/+} (WT), *Ku70*^{+/-}, and *Ku70*^{-/-} mouse embryo fibroblasts were incubated with a ³²P-labeled double-stranded oligonucleotide, 5'-GGGCCAAGAATCTTCCAGCAGTTTCGGG-3' (SEQ ID NO: 27). The protein-bound and free oligonucleotides were electrophoretically separated on a 4.5% native polyacrylamide gel. Gel slabs are dried and autoradiographed with Kodak X-Omat film.

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On page 27, lines 1-²⁴~~23~~, please delete the paragraph which begins "To determine whether a null mutation . . ." and insert the following paragraph:

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To determine whether a null mutation in *Ku70* affects the recombination of antigen-receptor genes in T and B lymphocytes *in vivo*, we measured the immunoglobulin and T-cell antigen receptor (TCR) rearrangements by PCR. DNA from bone marrow was amplified with primers specific to immunoglobulin D-J_H and V-DJ_H rearrangements, and DNA from thymus was amplified with primers that detect V-DJ_β and D_β-J_β-rearrangement (20, 25-28). Oligonucleotides for probes and PCR primers specific to TCR V_β-J_β rearrangements and immunoglobulin D-J_H and V-DJ_H rearrangements are as follows. For TCR_β V_β8-J_β2 rearrangements (28): V_β8.1: 5'-GAGGAAAGGT-GACATTGAGC-3' (SEQ ID NO: 28), J_β2.6: 5'-GCCTGGTGCCGGGACCGAAGTA-3' (SEQ ID NO: 29), V_β8 probe: 5'-GGGCTG AGGCTG ATCCATTA-3' (SEQ ID NO: 30). For D_β2-J_β1 rearrangement (20, 27): DR6: 5'-TGGCTTGACATGCAGAAAACACCTG-3' (SEQ ID NO: 31), DR53: 5'-TGAATTCCACAG-TCACTTGGCTTC-3' (SEQ ID NO: 32), and DR2 probe: 5'-GACACGTGATACAAAGCCCAGGGAA-3' (SEQ ID NO: 33). For immunoglobulin D-J_H and V-DJ_H rearrangements (26): 5'D: 5'-GTCAAGGGATCTACTACTGTG-3' (SEQ ID NO: 34), V7183: 5'-GAGAGAATTCAGAGACAATC-CCAAGAACACCCTG-3' (SEQ ID NO: 35), VJ558L: 5'-GAGAGAATTCTCCTCCAGCACAG-CCTACATG-3' (SEQ ID NO: 36), J2: 5'-GAGAGAATTCGGCTCCCAATGACCCTTTCTG-3' (SEQ ID NO: 37), 5'IVS: 5'-GTAAGAATGGCCTCTCCAGGT-3' (SEQ ID NO: 38), 3'-IVS: 5'-GACTCAATCACTAAGACA-GCT-3' (SEQ ID NO: 39), and probe: a 6 kb EcoR I fragment covering the J region of

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mouse IgM.

On page 62, lines 23-34, please delete the text which begins "The genotypes of the mice were first determined . . ." and insert the following text:

The genotypes of the mice were first determined by tail PCR analysis which distinguishes endogenous from the targeted Ku70 allele, and subsequently confirmed by Southern blot analysis. The PCR reaction contained 1 mg genomic DNA; 0.6 mM (each) of primers HO-2: GGGCCAGCTCATTCCTCCACTCATG (SEQ ID NO: 40), HO-3: CCTACAGTGTACCCGGACCTATGCC (SEQ ID NO: 25) and HO-4: CGGAACAGGACTGGTGGTTGAGCC (SEQ ID NO: 41); 0.2 mM (each) dNTP; 1.5 mM MgCl₂ and 2.5 U of Taq polymerase. Cycling conditions were 94°C for 1 min, 64°C for 1 min, 72°C for 1 min (30 cycles), followed by an extension at 72°C for 10 min. Primers HO-2 and HO-4 give a product of the targeted

On page 87, lines ¹⁶~~17~~-25, please delete the paragraph which begins "The genotype of the mice was determined by PCR . . ." and insert the following paragraph:

The genotype of the mice was determined by PCR which distinguishes endogenous from the targeted DNA-PKcs allele. PCR reaction contains 1 µg genomic DNA; 0.6 µM (each) of primers MD-20: TATCCGGAAGTCGCTTAGCA-TTG (SEQ ID NO: 42); MD-21: AAGACGGTTGAAGTCAGAAGTCC (SEQ ID NO: 43); and POL-8: TTCACATACACC-TTGTCTCCGACG (SEQ ID NO: 44); 0.2 mM(each) dNTP; 1.5 mM MgCl₂ and 2.5U of Taq polymerase. Primers

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MD-20 and MD-21 give a product of wild type allele that is 264 bp; primers MD-20 and Pol-8 yield a product of the targeted allele that is 360 bp.

On page 88, lines ⁶7-19, please delete the paragraph which begins "For RT-PCR assay, total RNA was prepared . . ." and insert the following paragraph:

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For RT-PCR assay, total RNA was prepared from SV40 transformed lung fibroblast cells using Qiagen RNeasy kit (Qiagen Inc., Santa Clarita, CA). After digestion of contaminated genomic DNA by DNase I (Ambion, Austin TX), cDNA synthesis was carried out with the Superscript preamplification system (Gibco BRL, Gaithersburg, MD) according to the included protocol. PCR primers used for RT-PCR were MD-3: ATCAGAAGGTCTAAGGCTGGAAT (SEQ ID NO: 45), MD-5: CGTACGGTGTGGCTACTGC (SEQ ID NO: 46) for amplification between exon 1 and 4 of DNA-PKcs, MD-28: CACTGAGGGCTT-TCCGCTCTTGT (SEQ ID NO: 47), MD-29: GCTCTTGTGCACGAATGTTGTAG (SEQ ID NO: 48) for PI-3 kinase domain, and GA-5: AGAAGACTGTGGATGGCCCC (SEQ ID NO: 49), GA-3: AGGTCCACCACCC-TGTTGC (SEQ ID NO: 50) for control GAPDH amplification.

On page 89, line ²¹~~22~~ - page 90, line 12, please delete the paragraph which begins "T cell antigen receptor (TCR) . . ." and insert the following paragraph:

T cell antigen receptor (TCR) and immunoglobulin recombination in T and B lymphocytes were measured by